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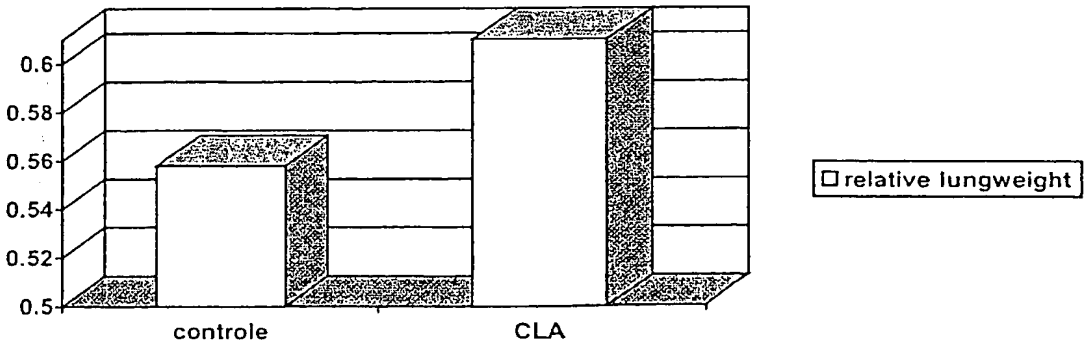
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: USE OF CONJUGATED LINOLEIC ACID DERIVATIVES



(57) Abstract: The invention concerns the use of conjugated linoleic acid (=CLA) or derivatives thereof, such as partial glycerides or triglycerides, alkyl esters or salts for the production of a food, a food supplement or a pharmaceutical preparation with the property to prevent or to cure influenza, to boost the effects of an influenza vaccination and / or to alleviate the effects of an influenza vaccination in humans and / or animals.

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USE OF CONJUGATED LINOLEIC ACID DERIVATIVES

This invention concerns in its broadest sense the use of Conjugated Linoleic Acid (= CLA) derivatives as an additive or as a component for foods, food supplements or pharmaceutical preparations which provide these foods, food supplements or pharmaceutical preparations with a specific health effect. From US 6 020 376 it is known that CLA can be used to maintain or to elevate CD-4 and CD-8 cell levels in animals to boost or benefit their immune system. However this document does not reveal a direct link with what type of disease can benefit from this use of CLA. As a certain ratio of CD-4 and CD-8 cells plays a role in the occurrence and / or treatment of many different types of diseases it remains unclear whether all these types of diseases can be prevented / cured by the use of CLA. This document further reveals that CLA can be used to alleviate the weight loss and other adverse effects from the production and exogeneous administration of TNFa in animals or humans or from the infection of animals or humans by viruses. According to column 9 lines 1 to 15 CLA can be used against infections by a number of different families of viruses. Although it is known that one group member of one of the viruses mentioned (= Orthomyxovirus family) comprise also the influenza virus genus, it cannot be derived from this document that CLA will have beneficial activity against influenza infections because other family members or serogroups of the same family are known to have other activities. Orthomyxovirus consists of two family members i.e. Thogoto like viruses and influenze viruses. Thogoto like viruses include Thogoto viruses, Dhori viruses and Batken viruses. These three genera generally are considered as three serogroups of the Thogoto

like viruses. They clearly differ from influenza viruses on the following points:

- Thogoto like viruses are transmitted via ticks and some vertebrates , the influenza viruses are transmitted in droplets as people sneeze , cough or talk , facilitated by close contact
- the mechanism of actions differ for the Thogoto like viruses from that of the influenza viruses. In contrast to the influenza viruses the Thogoto viruses are not applying the classical cap snatching mechanism
- the nature of the illness caused by the different viruses is different. Thogoto viruses leading to a more severe illness than influenza viruses such as optic neuritis and fatal meningitis.

Because of these basic differences in mechanism a man skilled in the art never would have expected that CLA could have a positive effect on all the family members of the whole Orthomyxovirus family.

US 5 827 885 being the mother patent of above US 376 has a similar teaching although the claims now are limited to the anti viral effects of CLA. Again influenza is not disclosed in this document. Here the same argument as above will account.

According to XP-002217392 (publication from Kelley c.s in Lipids 35 , no 10 , 2000 , pages 1065 - 1071) CLA has an immunizing activity on animals which however was not found to exist for healthy young women. Therefore this publication is teaching away from using CLA or its derivatives for use as anti influenza agent for humans.

US 5 674 901 discloses a similar teaching as above two US patents although the claims are limited to maintenance of CD-4 and CD-8 cell levels. So again no teaching is given that CLA would be beneficial against influenza.

As influenza is a known disease for already a very long time and no good means are known to prevent influenza, apart from an inactive influenza vaccination (for which humans sometimes are not always sensitive so that they develop as a reaction hereon insufficient amounts of anti-bodies to prevent them from suffering from influenza) there exists a great need to find compounds that would help to a higher extent than known so far to prevent / cure / treat humans suffering from influenza . A particular benefit is obtained by the fact that we found that the effects of an inactive influenza vaccination can be boosted by our compounds i.e., humans after influenza vaccination who also use in combination therewith our components will develop anti-bodies against influenza to a greater extent and in a shorter time than humans which are injected only.

Therefore our invention concerns in the first instance the use of conjugated linoleic acid (=CLA) or derivatives thereof, such as partial glycerides or triglycerides, alkyl esters or salts, wherein the CLA or derivative thereof is used for the production of a food, a food supplement or a pharmaceutical preparation with the property to prevent or to cure influenza, to boost the effects of an influenza vaccination and / or to alleviate the effects of an influenza vaccination in humans. The food that is made by our invention can contain a wide range of amounts of CLA. In practice effective amounts will be present in the food. Effective being defined as that amount that gives a noticeable positive effect. We however prefer to make foods that comprise from 0.1 to 20 grams of CLA derivative

per daily portion. So if the daily portion is just one item of food this item will contain the total amount of CLA, if however the daily portion is used in different portion during the whole day each portion will contain the reciprocal amount of CLA. It will be obvious from above that the CLA derivative is used for the production of a food, a food supplement or a pharmaceutical preparation with the ability to prevent or to cure influenza, caused by a virus of the influenza virus genus including the different serogroups.

Although about every type of food product can be made according to the invention we have a preference for the production of a food selected from the group consisting of margarine; fat continuous or water continuous or bicontinuous spreads, fat reduced spreads, confectionery products such as chocolate or chocolate coatings or chocolate fillings or bakery fillings; ice creams; ice cream coatings; ice cream inclusions; dressings, mayonnaises, cheeses, cream alternatives, dry soups, drinks, cereal bars, sauces, snack bars, dairy products, clinical nutrition and infant formula and having the ability to prevent or to cure influenza caused by a virus, in particular by a virus from the influenza virus genus including the different serogroups.

In the alternative we also can formulate our new invention as a method for administering a CLA derivative to a human suffering from influenza or to a human having the intention to prevent influenza by administering to this human an effective daily dosage of a CLA derivative (free acid / mono-, di- or tri-glycerides / alkyl esters, for example wherein the alkyl group contains from 1 to 12, more preferably 1 to 6, carbon atoms / salts, for example sodium salts). We have a preference for a method wherein the CLA derivative is administered in the form

of a food or a food supplement comprising an effective amount of CLA per portion, in particular 0.1 to 20 gram per daily portion. In particular we prefer a method wherein the CLA derivative is administered to a human suffering from influenza or trying to prevent influenza caused by a virus from the influenza virus genus including the different serogroups by administering an effective daily dosage of CLA-derivative per portion, in particular 0.1 to 20 gram per daily portion. The method may comprise a method of treating and/or preventing influenza.

Although we can use the different known isomers of CLA we prefer to use the isomers cis9trans11 and trans10cis12 and in particular mixtures of these isomers wherein the isomers are present in ratios of 80:20 to 20:80 and most preferably isomer mixtures wherein the trans10cis12 isomer is present for more than 60 wt %

In the instance that a food supplement is administered we prefer to supply the food supplement as a capsule in the form of a soft gel or a hard capsule wherein the encapsulating material comprises a material selected from the group consisting of gelatine; starch; modified starch; modified starch flour, sugars, in particular sucrose; lactose and fructose.

In the instance that a pharmaceutical preparation is administered we prefer to supply the pharmaceutical preparation in the form of a tablet, capsule, solution or emulsion depending upon the form of CLA employed and the route of administration.

According to a last embodiment of our invention we found that the CLA derivatives are most active when administered to elderly women (i.e., women with an age of at least 55 years) or to men.

Examples

Example 1

The resistance inducing effect of CLA against viral airway infections (prevention) was investigated / evaluated in rats. Rats were fed with CLA for 5 weeks before exposure (intranasal) to the influenza virus. The route of exposure is similar to the route humans are exposed to the influenza virus. The samples taken at several time points reflect the development of the infection and the protective effect by CLA.

Dosage= 1% CLA (FFA)
The following parameters were measured:

- growth
- food intake
- viral particles in lungs
- Influenza specific antibodies
- Th1 (IFN-gamma) and Th2 (IL10) cytokines
- Macrophage cytokine (IL1)

The results are shown in Figures 1, 2 and 3.

Figure 1 shows that for rats fed with CLA lungweight is increased due to infiltration of immune cells from the immune organs (for example the spleen) to the target (lung). The

immune cells can attack the virus (directly via macrophages or indirectly as initiated via cytokines).

Figure 2 shows the effect on the spleen. The spleen is a central immune organ. The spleen will increase in weight due to the increased amount of immune cells which are activated. Those immune cells will migrate to the target (lung) due to the infection.

Figure 3 shows the effect of CLA on influenza RNA. The influenza virus (expressed in viral particles (single-stranded RNA)) is cleared by the immune cells. This clearance occurs directly (macrophages) or indirectly (initiated by cytokines)

Example 2

Evaluation of the adjuvant effect of dietary supplementation with conjugated linoleic acid (CLA) on the immune response to influenza vaccine in healthy elderly subjects.

CLA was provided in capsules containing 1.25 g (1 g of active isomers) of a 50:50 mixture of the two isomers c9,t11 and t10, c12 CLA in either free fatty acid form (A-80) or triglyceride form (G-80). Placebo was provided in identical capsules containing high-oleic sunflower oil (HOSF). Two capsules (2 g of active isomer) were taken once daily with food for 49 days. Subjects were given a two week supply of individually packaged supplement or identical placebo on days 1, 14, 28, and 42.

The study was conducted on healthy, elderly (≥ 65 years old) adults.

The trial was conducted as a randomized, double-blind study of A-80, G-80, or control. All subjects received a single dose of inactivated influenza vaccine in open-label fashion.

The volunteers received either A80/G80 or HOSF for 28 days before vaccination. A single dose of inactivated influenza vaccine was administered intramuscularly on day 28 (± 3). The ability of CLA to adjuvant the immune response to influenza vaccine was assessed by comparison of the HAI antibody response to each of the three components of the vaccine between individuals who did or did not receive nutritional supplementation.

Subjects were classified as responders and non-responders for antibody production. For antibody production, subjects were considered responders when titers were above a certain titer as these levels are considered to be protective against Influenza infection.

Comparison of the effect of CLA in free fatty acid (A-80) or triglyceride (G-80) formulations

The overall response rates are shown in the table below

treatment	number of people	H1N1	H3N2	B
A80	22	50.0%	45.5%	90.0%
HOSF	27	33.3%	37.0%	63.3%
G80	23	21.7%	21.7%	78.3%

A vaccine contains 3 components (2 derived from Influenza A (expressed as H1N1 and H3N2), 1 from Influenza B)

The vaccination composition was:

H1N1: A/New Caledonia/20/99

H3N2: A/Moscow/10/99

B: B/Hong Kong/330/01

The conclusions that can be drawn from these results are:

- A80 increased the antibody response rates against all components of the influenza vaccination compared with control.
- G80 increased the antibody response rate against influenza B.

CLAIMS

1. Use of conjugated linoleic acid (=CLA) or derivatives thereof, such as partial glycerides or triglycerides, alkyl esters or salts, wherein the CLA or derivative thereof is used for the production of a food, a food supplement or a pharmaceutical preparation with the property to prevent or to cure influenza, to boost the effects of an influenza vaccination and / or to alleviate the effects of an influenza vaccination in humans.

2. Use according to claim 1 wherein the food made comprises 0.1 to 20 gram of CLA derivative per daily portion.

3. Use according to claims 1 or 2, wherein the CLA derivative is used for the production of a food or a food supplement with the ability to prevent or to cure influenza, caused by a virus of the influenza virus genus including the different serogroups.

4. Use according to claims 1, 2 or 3 wherein the CLA derivative is used for the production of a food selected from the group consisting of margarine; fat continuous or water continuous or bicontinuous spreads, fat reduced spreads, confectionery products such as chocolate or chocolate coatings or chocolate fillings or bakery fillings; ice creams; ice cream coatings; ice cream inclusions; dressings, mayonnaises, cheeses, cream alternatives, dry soups, drinks, cereal bars, sauces, snack bars, dairy products, clinical nutrition and infant formula and having the ability to prevent or to cure

influenza caused by a virus, in particular by a virus from the influenza virus genus including the different serogroups.

5. Method for administering a CLA derivative to a human suffering from influenza or to a human having the intention to prevent influenza by administering to this human an effective daily dosage of a CLA derivative (free acid / glycerides / alkyl esters / salts).

6. Method according to claim 5 wherein the CLA derivative is administered in the form of a food or a food supplement comprising an effective amount of CLA per portion, in particular 0.1 to 20 gram per daily portion.

7. Method according to claims 5 or 6 wherein the CLA derivative is administered to a human suffering from influenza or trying to prevent influenza caused by a virus from the influenza virus genus including the different serogroups.

8. Method according to claims 5, 6 or 7 wherein the effective dose that is administered is 0.1 to 20 g per day.

9. Method according to claim 6 wherein the food supplement is a capsule in the form of a soft gel or a hard capsule wherein the encapsulating material is selected from the group consisting of gelatine; starch; modified starch; modified starch flour, sugars, in particular sucrose; lactose and fructose.

10. Method according to claim 5 wherein the pharmaceutical preparation is in the form of tablet, capsule, solution or

emulsion depending upon the form of CLA employed and the route of administration.

11. Method according to claims 5 to 10 wherein the CLA derivative is administered to elderly women (i.e women having an age of 55 years or more) or to men

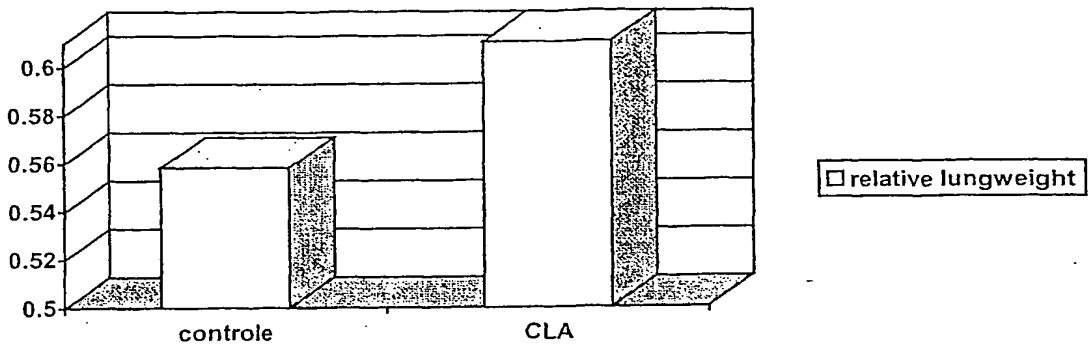

Fig.1

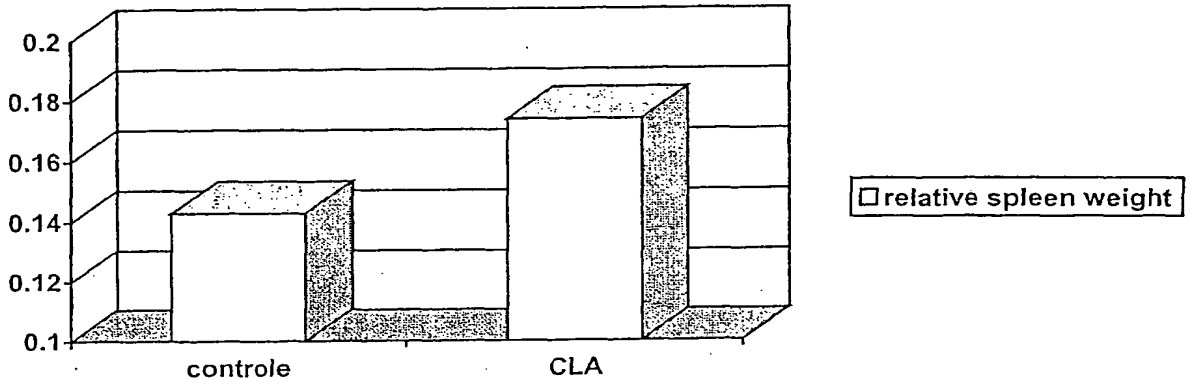

Fig.2

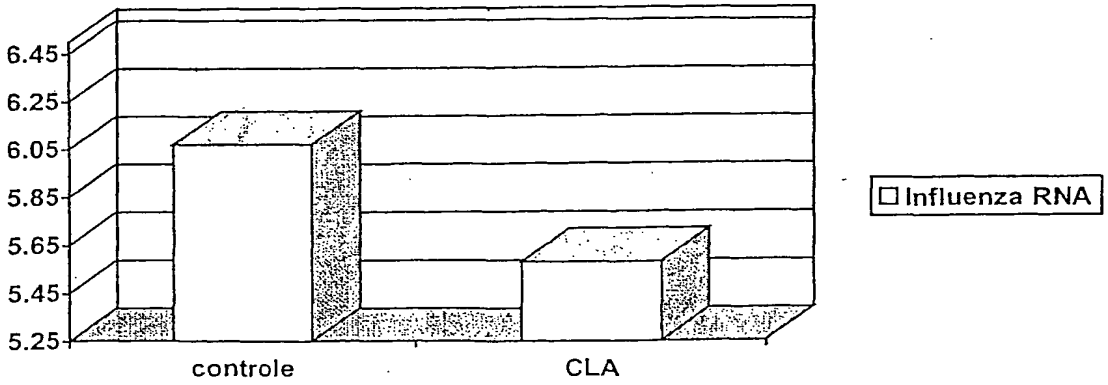

Fig.3

INTERNATIONAL SEARCH REPORT

Internat l Application No
PCT/GB 03/01726

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/20 A23L1/30 A61K31/201

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, FSTA, BIOSIS, MEDLINE, CHEM ABS Data, PHARMAPROJECTS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 827 885 A (COOK MARK E ET AL) 27 October 1998 (1998-10-27) cited in the application column 8, line 44 -column 10, line 2; claim 3	1-11
X	WO 00 67596 A (TUFTS COLLEGE ;MEYDANI MOHSEN (US); MEYDANI SIMIN NIKBIN (US)) 16 November 2000 (2000-11-16) page 3, line 8-13; claims 1,12 page 6, line 5-27 page 16, line 7-23	1-11
X	EP 0 396 251 A (UNIV SINGAPORE) 7 November 1990 (1990-11-07) page 3, line 4-13; claims 6,7 page 3, line 34 -page 4, line 3	1-11

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 July 2003	01/08/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040. Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Heirbaut, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/GB 03/01726

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; October 2000 (2000-10) KELLEY D S ET AL: "Dietary conjugated linoleic acid did not alter immune status in young healthy women." Database accession no. PREV200000541823 XP002217392 abstract & LIPIDS, vol. 35, no. 10, October 2000 (2000-10), pages 1065-1071, ISSN: 0024-4201 ---	1-11
X	BASSAGANYA-RIERA J ET AL: "Long-term influence of lipid nutrition on the induction of CD8responses to viral or bacterial antigens" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 20, no. 9-10, 31 January 2002 (2002-01-31), pages 1435-1444, XP004334125 ISSN: 0264-410X abstract page 1442, column 1, paragraph 2 -page 1143, column 1, paragraph 3 ---	1-11
X	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 22 March 2002 (2002-03-22) ALBERS RUUD ET AL: "The immunomodulatory effects of conjugated linoleic acid (CLA) in human volunteers." Database accession no. PREV200200370185 XP002217397 abstract -& FASEB JOURNAL, vol. 16, no. 5, 22 March 2002 (2002-03-22), page A983 XP002217670 Annual Meeting of Professional Research Scientists on Experimental Biology;New Orleans, Louisiana, USA; April 20-24, 2002, March 22, 2002 ISSN: 0892-6638 page 983, column 1-2, paragraph 725.5 -----	1-11

INTERNATIONAL SEARCH REPORT

Int ional application No.
rCT/GB 03/01726

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 5-11
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 5-11 are directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the composition.

Continuation of Box I.1

Claims Nos.: 5-11

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat Application No
PCT/GB 03/01726

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